36. (New) The DNA delivery adenovirus vector according to claim 30 or claim 34 wherein the repressor binding site is a E2F binding site.

37. (New) The DNA delivery adenovirus vector according to claim 29 further comprising a heterologous gene operatively linked to a promoter.

REMARKS

Applicants respectfully request favorable reconsideration in view of the herewith presented amendment and remarks.

No new matter has been introduced by the Substitute Sequence Listing and the amendments made to the specification. The amendments made to the specification only encompass the introduction of SEQ ID NO identifiers. The Substitute Sequence Listing differs from the originally-filed Sequence Listing in format and at SEQ ID NOs:12, 16 and 31. The originally-filed Sequence Listing contained inadvertant errors in the sequence for SEQ ID NOs:12, 16 and 31. Support for the corrected sequence shown in the Substitute Sequence Listing for SEQ ID NOs:12, 16 and 31 is found in the specification at: page 27, line 17 for SEQ ID NO:12; page 30, lines 25-27 for SEQ ID NO:16; and Figure 10C for SEQ ID NO:31.

The amendment to claim 1 corresponds to the Examiner's suggestions in the Official Action dated March 27, 2002 (see page 9). The changes for claim 1 are requested in order to place claim 1 in better form. Support for these amendments is found at page 16, lines10-20. Further, the amendment to step (a) of claim 1 is supported throughout the specification, for example, at page 5, line 30, to page 6, line 7.

The amendment of step (b) of claim 1 is supported in the specification, for example, at page 17, lines 22-25; at page 20, lines 28-35; and at page 10, line s 24-34. Further, support for the Examiner's suggested recitation "a promoter operatively linked to the heterlogous gene", (see page 6, lines 9-10, of the Official Action dated March 27, 2002), is found at page 17,

line 25, to page 18, line 5. Lastly, support for the recitation "a second adenovirus packaging sequence" is found at page 17, lines 4-7.

Support for the amendments made to step (c) of claim 1 is found throughout the specification, for example, at page 18, line 26, to page 19, line 11. Support for the amendments made to step (d), which involve repressing packaging of the DNA delivery adenovirus vector, is found throughout the specification, for example, in the paragraph beginning at page 19, line 18. This section of the instant specification refers to either helper or DNA delivery adenovirus vectors, and thus supports new claim 21, which is identical to claim 1 except step (d) involves repressing packaging of the DNA delivery adenovirus vector. Another passage in the specification which refers to the versatility of packaging repression is found at page 7, lines 6-12: "Optionally, the system can be designed to allow efficient packaging of one adenovirus vector while inhibiting packaging of a different vector in the same infected cell by using viruses with different packaging sites and/or COUP-TF binding sites in conjunction with COUP-TF over-expression."

The changes to dependent claims 2 and 3, and the addition of new claims 22 and 23 (which depend on claim 21), reflect the changes made in claim 1. Specifically, these dependent claims are directed to a particular repressor which binds to the repressor binding site contained in either the helper adenovirus vector or the DNA delivery vector.

The amendments to claims 4 and 5, and the addition of new claims 24 and 25, are supported in the specification, for example, at page 6, lines 29-35.

Claims 6 and 7 have been amended to specify that the adenovirus vector may be a helper adenovirus vector. New claims 26 and 27 are identical to claims 6 and 7, except that claims 26 and 27 specify that the adenovirus vector is a DNA delivery adenovirus vector. Claim 28 multiply depends on claims 26 and 27, and specifies that DNA delivery adenovirus vectors further comprise a promoter that is operatively linked to a heterlogous gene. Support for claim 28 is found in original claim 8. Support for a DNA delivery vector comprising a promoter operatively linked to a heterologous gene is found, for example, at Figure 8A. Further, the specification states that many different types of promoters may be used, for example, on page 17, line 35, to page 18, line 5.

Claim 9 has been amended in accordance with the Examiner's suggestions. Claims 10-17 have been amended to specify that the adenovirus vectors are helper adenovirus vectors. Claims 29-36 are identical to claims 10-17 respectively, except that claims 29-36 specify that the adenovirus vectors are DNA delivery adenovirus vectors. Claim 37 is identical to original claim 18, except that claim 37 specifies that the adenovirus vector is a DNA delivery adenovirus vector and that the DNA adenovirus vector further comprises a promoter operatively linked to a heterlogous gene. Claim 19 has been amended to be dependent upon the method of claim 9.

No new matter has been introduced by the claim amendments. Applicants respectfully request entry and consideration of the amendments.

Response to Claim Rejections Under 35 USC §101

Claims 8 and 18, and dependent claims therefrom, have been rejected under 35 USC §101 because the Examiner contends that these claims are not supported by a well-asserted or established utility. Specifically, the Examiner argues: "that a helper virus comprising of a heterologous gene would require further research to identify and reasonably confirm a 'real world' context of use and therefore, a substantial utility is not defined." Applicants respectfully disagree with this rejection. However, in order to expedite prosecution of the pending claims, applicants have cancelled claims 8 and 18. Further, new claims 28 and 37 which correspond to claims 8 and 18 respectively, are in a form which addresses the Examiner's concerns. Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

Response to Claim Rejections Under 35 USC §112, First Paragraph

The claims have been rejected under 35 USC §112, First Paragraph, because the Examiner states claims 1-19 are not enabled. Applicants respectfully disagree with this rejection. However, in order to facilitate prosecution of the pending claims, applicants have amended the claims to address the Examiner's concerns. Reconsideration and withdrawal of this ground of rejection is respectfully requested.

Response to Claim Rejections Under 35 USC §112, 2nd Paragraph

The Examiner has rejected claim 8-18 because these dependent claims used the article 'an' instead of 'the'. Applicants have corrected this inadvertent error and therefore respectfully request withdrawal of this ground of rejection.

Allowance of the pending claims is respectfully requested. Early and favorable action by the Examiner is earnestly solicited.

AUTHORIZATION

No additional fee is believed to be necessary.

The Commissioner is hereby authorized to charge any additional fees which may be required for this amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 3927-4133US2.

Respectfully submitted

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Dated: June 25, 2002

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APPENDIX

(Additions are indicated by underlined text and deletions are indicated by [bracketed text].

The amendments requested for the Specification only entail the introduction of SEQ ID NO: identifiers:

The paragraph on page 5, lines 10-29, has been amended as follows:

The present invention relates to adenovirus vectors containing a minimum packaging signal for producing adenovirus virions. Of special importance is the presence of a CG dinucleotide located downstream of a TTTG sequence within each of the packaging elements. Spacing between the consensus segment 5'-TTTG-3' and the 5'-CG-3' segment located downstream is preferably between 1 and 12 nucleotides. Alternatively, it amy be preferred to configure the consensus segments so that these elements appear on the same surface of the DNA helix. Most preferably, the adenovirus vector of the present invention may contain a packaging element consisting of 5'-TTTGN$_8$CG-3' (SEQ ID NO:1) which represents a minimal sequence necessary for adenovirus packaging. This sequence is preferably present in multiple copies. One type of minimal packaging sequence is an "A repeat", which contains a consensus sequence. Several A repeat sequences are shown in Table 1.

The paragraph on page 8, lines 1-22, has been amended as follows:

FIG. 1 depicts the adenovirus type 5 packaging domain. (A) A schematic representation of the left end of the adenovirus type 5 genome. Nucleotide positions are indicated by numbers. The inverted terminal repeat (ITR) is represented by a gray box. Viral packaging repeats are termed A repeats I to VII (arrows). The E1A transcriptional start site is indicated by an arrow, and enhancer elements I and II are designated E1A enhancer. (B) The packaging repeat consensus motif. Shown is an alignment of A repeats I (SEQ ID NO:3), II (SEQ ID NO:4), V (SEQ ID NO:7) and VI (SEQ ID NO:8). Nucleotides comprising the

bipartite consensus motif for A repeats I, II, V and VI are boxed and enlarged. The consensus motif is shown at the bottom (5'-TTTGN$_8$CG-3' (SEQ ID NO:1)). (C) Alignment of A repeats V and VI in different adenovirus subgroups: Ad 5 (subgroup C) (SEQ ID NO:23), Ad 4 (subgroup E) (SEQ ID NO:24), Ad 12 (subgroup A) (SEQ ID NO:25), Ad 3 (subgroup B) (SEQ ID NO:26), Ad 9 (subgroup D) (SEQ ID NO:27). The positions of AV and AVI are shown by horizontal lines above the sequence. Nucleotides identical between all subgroups are indicated by vertical lines.

The paragraph on page 10, lines 9-23, has been amended as follows:

FIG. 7 depicts multimerized oligonucleotides corresponding to A repeats AI (SEQ ID NO:28) and AVI (SEQ ID NO:29) used to construct recombinant viruses. A dimeric oligonucleotide sequence is shown to simplify the schematic diagram. The potential COUP-TF binding sites in the oligonucleotides are indicated by arrows. Perfect or 4-out-of-5 nucleotide matches to the COUP-TF consensus sequence are shown as closed arrowheads; 3-out-of-5 nucleotide matches to the COUP-TF consensus site are shown as open arrowheads. Perfect, or nearly-perfect, COUP-TF binding sites with a 1 base spacing are found in multiple locations in the AVI oligonucleotide repeat, but not in the AI oligonucleotide repeat.

The paragraph on page 12, lines 6-25 has been amended as follows:

Fig. 10 depicts synthetic oligonucleotides that contain different adenovirus packaging repeats designed with specific repressor binding sites that either overlap the packaging A repeats or are placed between packaging A repeats. (A) The sequence of the wild type AV-AVII oligonucleotide (SEQ ID NO:16). A dimeric copy of this oligonucleotide efficiently directed packaging in a recombinant virus (Fig. 2). A repeats V, VI and VII are indicated and the consensus packaging repeats are encircled. (B) The AV-AVII oligonucleotide is modified (SEQ ID NO:30) (underlined nucleotides) to create a high affinity binding site for the adenovirus-induced E2F-E4-6/7 protein complex overlapping A repeats V and VI (binding site

indicated by inverted arrows). (C) The AV-AVII oligonucleotide is modified (SEQ ID NO:31) (underlined nucleotides) to create a high affinity binding site for the *E. coli* lac repressor overlapping and adjacent to A repeat V (binding site indicated by inverted arrows).

The paragraph beginning on page 14, line 27, and ending on page 15, line 22, has been amended as follows:

The present invention also relates to the identification of a minimum adenovirus packaging signal. A minimal packaging sequence of 5'-TTTGN_8CG-3' (SEQ ID NO:1) has been identified. Although eight nucleotides are preferred to separate the left portion of the packaging consensus element (i.e., 5'-TTTG-3') from the right portion (i.e., 5'-CG-3'), this spacing may vary 1 to 12 nucleotides. Alternatively, it may be preferred to configure the consensus segments so that the segments appear on the same surface of the DNA helix. the packaging element may be inserted into the left or right end of the adenovirus vector, preferably within 600 nucleotides from either end. More preferably, this minimal sequence is present at the left end of the genome and is present in multiple copies. Another consensus sequence comprises 5'-ATTTGN_8CG-3' (SEQ ID NO:2) and provides a strong packaging signal in adenovirus vectors. Two copies of this minimal packaging sequence are sufficient to ensure packaging. More than two copies enhance virus packaging. However, any number of this sequence can be inserted into the virus to ensure particle production. "Multimerized" as this term is used in the instant application refers to multiple copies of an element (i.e. packaging or repressing). These elements may be present in single units or in multimers, which preferably means 2-36 repeats and more preferably 2-12 units or elements. One form of the minimal packaging element is an "A repeat", which is derived from adenovirus. Representative A repeats are set forth below in Table 1:

Table 1 on page 15 has been amended as follows:

TABLE 1

AI:	5'-TTTGGGCGTAACCG-3' (SEQ ID NO:3)
AII:	5'-TTTGGCCATTTTCG-3' (SEQ ID NO:4)
AIII:	5'-TCTGAATAATTTTG-3' (SEQ ID NO:5)
AIV:	5'-TTTGTGTTACTCAT-3' (SEQ ID NO:6)
AV:	5'-TTTGTCTAGGGCCG-3' (SEQ ID NO:7)
AVI:	5'-TTTGACCGTTTACG-3' (SEQ ID NO:8)
AVII:	5'-TTTACGTGGAGACT-3' (SEQ ID NO:9)

The paragraph on page 19, lines 13-17, has been amended as follows:

Another important aspect of the present invention relates to gene therapy vectors that use adenovirus minimal packaging sequence, 5'-TTTGN_8CG-3' (SEQ ID NO:1). (See Provisional patent application no. 60/081,867, incorporated herein by reference).

The paragraph that begins on page 23, line 29, and ends on page 24, line 16, has been amended as follows:

Cellular P-complex is a bona-fide adenovirus packaging component. This complex appears to contain a TATA binding protein (TBP) and a second protein called TAF172 (Timmers et al. 1992, Taggart et al. 1992). P-complex binding is inhibited by ATP and magnesium. Complex formation is observed on all minimal packaging domains that exhibit functional activity *in vivo*. The affinity of the P complex for the different multimeric A repeats *in vitro* correlates well with the ability of the respective cis-acting sequences to support viral DNA packaging *in vivo*. Specifically, AI and AV-VII constitute strong P complex binding sites and they confer maximal packaging activity *in vivo*. The most preferred P-complex binding sites

comprise a hexamer of AI and a dimer of AV, AVI and AVII. On the other hand, AVI is noted as a weak binding site for P complex *in vitro*, and it serves as a particularly weak packaging domain *in vivo*. As discussed above, the Ad packaging consensus motif is a bipartite sequence with a conserved AT-rich and a GC-rich half site (5'-TTTGN$_8$CG-3' (SEQ ID NO:1)) (Schmid, et al. (1997)).

The paragraph on page 27, lines 3-34, has been amended as follows:

Virus constructions. Ad5 d1309, the parent for all the viruses described in this report, is a phenotypically wild type virus that contains a unique XbaI cleavage site at 3.8 map units (Jones, et al. (1979)). Plasmid pE1A-194/814 contains the left end Ad5 XbaI fragment (nt 1-1339) with a deletion between nt 194 and 814 and a unique XhoI restriction site at the junction of the deletion. A head-to-tail hexamer of an oligonucleotide containing A repeat VI (5'-TCGACCGCGGGGACTTTGACC-3' (SEQ ID NO:10):5'-TCGAGGTCAAAGTCCCCGCGG-3' (SEQ ID NO:11)) was cloned into the 194/814 deletion. Similarly, head-to-tail hexamers of oligonucleotides containing A repeat I (5'-TCGAGTTGTAGTAAATTTGGG-3' (SEQ ID NO:12):5'-TCGACCCAAATTTACTACAAC-3' (SEQ ID NO:13)) or A repeat II (5'-TCGACCGAGTAAGATTTGGCC-3' (SEQ ID NO:14):5'-TCGAGGCCAAATCTTACTCGG-3' (SEQ ID NO:15)) were cloned into the pE1A-194/814 background. pBR-194/814 and pBR-53/814 have sequences between nt 194 and 814 and nt 53 and 814 deleted. A monomer and dimer of viral sequences is located between nt 334 and 385 which contain AV, AVI, and AVII was cloned into the 194/814 deletion. A dimer of the nt 334 to 385 fragment as well as 12 head-to-tail copies of an oligonucleotide containing AVI (5'-TCGACCGCGGGGACTTTGACC-3' (SEQ ID NO:10):5'-TCGAGGTCAAAGTCCCCGCGG-3' (SEQ ID NO:11)) were cloned into the 53/814 deletion in either orientation. All mutations were verified by nucleotide sequence analysis.

The paragraph beginning on page 30, line 16, and ending on page 31, line 3, has been amended as follows:

Plasmids, probes and competitor fragments. Head-to-tail hexamers of A repeats I and VI, individually, and a dimer of A repeats V-VII were cloned into pUC9. The sequence of a monomer of A repeat I is: 5'-TCGAGTTGTAGTAAATTTGGG-3' (SEQ ID NO:12): 5'-TCGACCCAAATTTACTACAAC-3' (SEQ ID NO:13), a monomer of A repeat VI is: 5'-TCGACCGCGGGGACTTTGACC-3' (SEQ ID NO:10): 5'-TCGAGGTCAAA GTCCCCGCGG-3' (SEQ ID NO:11), a monomer of AV-AVII is: 5'-TCGACCGCGTAATATTTGTCTAGGGCCGCGGGGACTTTGACCGTTTACGTGGAGACT CC-3' (SEQ ID NO:16): 5'-TCGAGGAGTCTCCACGTAAACGGTCAAAGTCCCCGCGGC CCTAGACAAATATTACGCGG-3' (SEQ ID NO:17). The fragments were liberated from the vector by digestion with EcoRI and HindIII, gel purified and ^{32}P-end-labeled with Klenow DNA polymerase and (α-^{32}P)dATP. For the preparation of ITR 1-13 probe, a monomeric oligonucleotide representing the left end 13 nt flanked by Xho/Sal linkers (5'-TCGACATCATCAATAATC-3' (SEQ ID NO:18): 5'-TCGAGATTATTGATGATG-3' (SEQ ID NO:19)) was end-labeled in the same way using (α-^{32}P)dCTP.

The paragraph beginning on page 31, line 4, and ending on page 32, line 5, has been amended as follows:

For the preparation of competitor fragments containing packaging repeats, monomeric oligonucleotides were multimerized using T4 DNA ligase. Selection for head-to-tail multimers was achieved by subsequent digestion using SalI and XhoI, followed by phenol/chloroform extraction and ethanol precipitation. In addition to multimers prepared from the oligonucleotides representing packaging elements I, VI and V-VII described above, A repeat II (5'-TCGACCGAGTAAGATTTGGCC-3' (SEQ ID NO:14):5'-TCGAGGCCA AATCTTACTCGG-3' (SEQ ID NO:15)) and A repeat V (5'-TCGACCGCGTAATATTTGTCC-3' (SEQ ID NO:20):5'-TCGAGGACAAATATTACGCGG-

3' (SEQ ID NO:21)) were used as multimeric competitors. Packaging repeat competitor fragments designated LS have the underlined nucleotides shown above in AI, AII, AV, AVI, AV-VI mutated into the sequence 5'GTGCAG-3' (only the upper strand is indicated). the italicized CG dinucleotide in the AV competitor was replaced by an AT in the competitor fragment designated CG. The competitor oligonucleotide representing ITR sequences 1-13 was used in monomeric form and was identical to the one used for probe preparation. The monomeric ITR 10-22 competitor oligonucleotide contains sequences between Ad nt 10-22 flanked by XhoI/SalI linkers. Quantitation of oligonucleotide competitors was performed spectrophotometrically. The amount of specific competitor DNA added per binding reaction is indicated in the text as -fold molar excess of binding sites present in the competitor relative to binding sites present in the probe. This definition, however, is based on the assumption that one binding site (located between nt 1-13) is present in monomeric ITR fragments and that six binding sites are present in hexameric packaging repeat fragments.

Please replace the paragraph on page 39, lines 8-25, with the following:

COUP-TF interacts with adenovirus packaging elements. Database searches revealed that the AVI probe contains a highly conserved dimeric consensus binding sites for a cellular transcription factor, chicken ovalbumin upstream promoter transcription factor (COUP-TF; Cooney et al. (1992)). COUP-TF binds to the consensus sequence 5'-GGTCA-3' when situated as a direct or inverted repeat, with a preferred spacing of 1 base pair, and represented as perfect or imperfect versions of the consensus binding site. These binding sites overlap A repeat VI (5'-GGACTTTGACC-3' (SEQ ID NO:22); the COUP-TF inverted repeat is underlined, and AVI is in bold), only the upper strand is indicated with the COUP half sites underlined and AVI indicated in bold case. Other A repeats contain similar sequence motifs, albeit with less resemblance to the dimeric COUP consensus.

Claims 1-19 have been amended as follows:

1. A method of regulating adenovirus packaging comprising the steps of:

[a.](a) obtaining [an] a helper adenovirus vector containing [an] a first adenovirus packaging [repressor binding site] sequence comprising a repressor binding site, [said binding site being located between, within or surrounding an adenovirus packaging sequence; and]

[b.](b) obtaining a DNA delivery adenovirus vector comprising 5' and 3' inverted terminal repeats; a second adenovirus packaging sequence; a heterologous gene; and a promoter operatively linked to the heterologous gene;

(c) propagating [said] the helper adenovirus vector and the DNA delivery adenovirus vector [in the absence of said packaging repressor] in a cell-line; and

[c.] (d) repressing packaging of [said] the helper adenovirus vector [in the presence of said packaging] by a repressor which binds to the repressor binding site contained in the helper adenovirus vector.

2. The method [of] according to claim 1 wherein the [adenovirus packaging] repressor is COUP-TF.

3. The method [of] according to claim 1 wherein the [adenovirus packaging] repressor is *lac* repressor.

4. The method according to claim 1 wherein the propagating step occurs in a first cell-line and the [packaging] repressing step occurs in a second cell-line, wherein the repressing step in the second cell-line further comprises a step selected from the group of steps consisting of:

(a) endogenously expressing the repressor; and

(b) transfecting a vector expressing the repressor.

5. The method according to claim 1 wherein the [packaging] repressing step occurs in [a] the cell-line of step (c) and wherein the repressing step further comprises a step selected from the group of steps consisting of: [coinfected with a vector expressing the adenovirus packaging repressor]

(a) endogenously expressing a repressor; and

(b) transfecting a vector expressing the repressor.

6. [An] A helper adenovirus vector comprising an adenovirus packaging sequence containing a plurality of COUP-TF binding sites comprising an A repeat VI element.

7. [An] A helper adenovirus vector comprising an adenovirus packaging sequence having at least two copies of 5'-TTTGN$_8$CG-3' and a plurality of COUP-TF binding sites, comprising an A repeat VI element.

9. A method of administering a replicant defective adenovirus to a mammal comprising the steps of:

[a.](a) [encapsidating] packaging [the] a DNA delivery adenovirus vector [of claim 8, thereby forming an adenovirus] according to the method of claim 1;

[b.](b) isolating [said] the packaged DNA delivery adenovirus vector;

[c.](c) preparing [said] the packaged DNA delivery adenovirus vector in a pharmaceutically acceptable carrier; and

[d.](d) administering [said] the prepared and packaged DNA delivery adenovirus vector to [a] said mammal.

10 [An] A helper adenovirus vector [containing] comprising a packaging signal sequence consisting of at least two copies of 5'-TTTGN$_8$CG-3' (SEQ ID NO:1) and an A repeat VI element.

11. [An] The helper adenovirus vector according to claim 10 wherein [an adenovirus packaging] a repressor binding site is embedded in the packaging signal sequence.

12. [An] The helper adenovirus vector according to claim 10 wherein [an adenovirus packaging] a repressor binding site flanks the packaging signal sequence.

13. [An] The helper adenovirus vector according to claim 10 wherein [an adenovirus packaging] a repressor binding site alternates with the packaging signal sequence.

14. [An] The helper adenovirus vector according to claim 10 having 3-12 packaging signal sequences.

15. [An] The helper adenovirus vector according to claim 14 wherein [an adenovirus packaging] a repressor binding site is located between packaging signal sequences.

16. [An] The helper adenovirus vector according to claim 11 or 15 wherein the [adenovirus packaging] repressor binding site is a *lac* repressor site.

17. [An] The helper adenovirus vector according to claim 11 or 15 wherein the [adenovirus packaging] repressor binding site is a E2F binding site.

19. [A] The method of administering a replicant defective adenovirus to a mammal according to claim 9, wherein step (a) is conducted with a helper adenovirus according to claims 6, 7 or 10. [comprising the steps of:]

[a. encapsidating the adenovirus vector of claim 10, thereby forming an adenovirus;]

[b. isolating said adenovirus]

[c. preparing said adenovirus in a pharmaceutically acceptable carrier; and]

[d. administering said adenovirus to a mammal.]